IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Browning et al. Examiner: Unassigned

Serial No.: Unassigned Group Art Unit: Unassigned

(Divisional of 09/000,166)

Filing Date: On Even Date Herewith

For: METHODS FOR INHIBITING LYMPHOTOXIN BETA RECEPTOR SIGNALLING (As amended)

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

After granting a filing date in the above-referenced patent application and prior to calculation of the filing fee, kindly enter the following preliminary amendments and remarks:

IN THE TITLE:

The title has been amended to read:

-- METHODS FOR INHIBITING LYMPHOTOXIN BETA RECEPTOR SIGNALLING--

IN THE CLAIMS:

Claims 1-69 were originally pending in U.S.S.N. 09/000,166. Please delete claims 1-53 and 68-69 without prejudice or disclaimer of the subject matter contained therein. Applicants specifically reserve the right to file a divisional or continuation application to the subject matter contained in these.

IN THE SPECIFICATION:

In accordance with CFR 1.121, please amend the specification as follows:

On page 1, after the Title please insert:

--Related Applications--

--This is a divisional of U.S. national stage application filed under 35 U.S.C. 371 filed on July 19, 1996, which is a continuation-in-part application of U.S.S.N. 08/505,606 filed on July 21, 1995, now U.S. patent number 5,925,351. The entire disclosures of the aforesaid patent applications are incorporated herein by reference.--

On Page 14, line 35-page 15, line 8:

Soluble [**LT-α/â**] **LT-α/β** heteromeric complexes comprise [**LT-â**] **LT-β** subunits which have been changed from a membrane-bound to a soluble form. These complexes are described in detail in applicants' co-pending international application (PCT/US93/11669, published June 23, 1994 as WO 94/13808). Soluble [**LT-â**] **LT-β** peptides are defined by the amino acid sequence of [**lymphotoxin-â**] **lymphotoxin-β** wherein the sequence is cleaved at any point between the end of the transmembrane region (i.e. at about amino acid #44) and the first TNF homology region (i.e. at amino acid #88) according to the numbering system of Browning et al., Cell, 72, pp. 847-56 (1993).

On Page 15, lines 9-20

Soluble [**LT-â**] **LT-β** polypeptides may be produced by truncating the N-terminus of [**LT-â**] **LT-β** to remove the cytoplasmic tail and transmembrane region (Crowe et al., Science, 264, pp. 707-710 (1994)). Alternatively, the transmembrane domain may be inactivated by deletion, or by substitution of the normally hydrophobic amino acid residues which comprise a transmembrane domain with hydrophilic ones. In either case, a substantially hydrophilic hydropathy profile is created which will reduce lipid affinity and improve aqueous solubility. Deletion of the transmembrane domain is preferred over substitution with hydrophilic amino acid residues because it avoids introducing potentially immunogenic epitopes.

On Page 15, line 30 to page 16, line 2:

Soluble [**LT-α/â**] **LT-α/β** heteromeric complexes may be produced by co-transfecting a suitable host cell with DNA encoding LT-á and soluble [**LT-â**] **LT-β** (Crowe et al., J. Immunol. Methods, 168, pp. 79-89 (1994)). Soluble [**LT-â**] **LT-β** secreted in the

absence of LT-α is highly oligomerized. However, when co-expressed with LT-α, a 70 kDa trimeric-like structure is formed which contains both proteins. It is also possible to produce soluble **[LT-α1/â2]** **LT-α1/β2** heteromeric complexes by transfecting a cell line which normally expresses only LT-α (such as the RPMI 1788 cells discussed above) with a gene encoding a soluble **[LT-â]** **LT-β** polypeptide.

On Page 16, lines 3-6:

LT-α and **[LT-â]** **LT-β** polypeptides may be separately synthesized, denatured using mild detergents, mixed together and renatured by removing the detergent to form mixed LT heteromeric complexes which can be separated (see below).

On Page 16, lines 7-19:

Purification of **[LT-α1/â2]** **LT-α1/β2** Complexes

Soluble **[LT-α1/â2]** **LT-α1/β2** heteromeric complexes are separated from co-expression complexes comprising a different subunit stoichiometry by chromatography using TNF and **[LT-â]** **LT-β** receptors as affinity purification reagents. The TNF receptors only bind within α/α clefts of LT complexes. The **[LT-â]** **LT-β** receptor binds with high affinity to **[â/â]** β/ β clefts, and with lower affinity to [α/â] α/β clefts of heteromeric **[LT-α/â]** **LT-α/β** complexes. Accordingly, LT- α3 and **[LT-α2/â1]** **LT-α2/β1** will bind to TNF-R. The **[LT-â-R]** **LT-β-R** can also bind **[LT-α2/â1]** **LT-α2/β1** trimers (within the [α/â] α/β clefts) but cannot bind LT- α3. In addition, the **[LT-â-R]** **LT-β-R** (but not TNF-R) binds **[LT-α1/â2]** **LT-α1/β2** and **[LT-ân]** **LT-β(n)** (the exact composition of such preparation is unknown, however, they are large aggregates).

On Page 16, lines 28-34:

There are two schemes by which the **[LT-α1/â2]** **LT-α1/β2** ligand can be purified using receptors and immuno-affinity chromatography. In the first scheme, a supernatant from an appropriate expression system co-expressing both LT-α and the truncated **[LT-â]** **LT-β** form is passed over a TNF-R column. The TNF-R will bind LT- α3 and

[LT-α2/â1] LT-α2/β1 trimers. The flow through from the TNF-R column will contain **[LT-â(n)]** LT-β(n) and **[LT-α1/â2]** LT-α1/β2.

Page 16, line 35 to page 17, line 5:

In the second scheme, all **LT-â-containing** forms (**[LT-â(n)]** LT-β(n), **[LT-α1/â2]** LT-α1/β2 and **[LT-α2/â1]** LT-α2/β1) are bound to and eluted from a **[LT-â-R]** LT-β-R column using classical methods such as chaotrophe or pH change. (LT- α3 flows through this column). The eluate is neutralized or the chaotrophe removed, and the eluate is then passed over a TNF-R column, which binds only to the **[LT-α2/â1]** LT-α2/β1 trimers. The flow through of this column will contain **[LT-â(n)]** LT-β(n) and **[LT-α1/â2]** LT-α1/β2 trimers.

Page 17, lines 6-8:

In both cases, pure **[LT-α1/â2]** LT-α1/β2 trimers can be separated from **[LT-â]** LT-β by subsequent gel filtration and/or ion exchange chromatographic procedures known to the art.

Page 17, lines 9-13:

Alternatively, different forms of **[LT-α/â]** LT-α/β heteromeric complexes can be separated and purified by a variety of conventional chromatographic means. It may also be preferable to combine a series of conventional purification schemes with one of the immunoaffinity purification steps described above.

Page 47, line 28 to page 48 line 9:

Soluble mouse **[LT-α/â]** LT-α/β complexes were prepared as described above for the human soluble LT-α/ß complexes. The soluble mouse **[LT-â]** LT-β subunit was made based on sequence information previously described (Lawton et al., J. Immunol., 154, pp. 239-46 (1995)). Soluble murine **[LT-α/â]** LT-α/β complexes were expressed using the baculovirus/insect cell expression system and the **[LT-α/â]** LT-α/β complexes were isolated by affinity chromatography using human p55 TNF-R and **[LT-â-R]** LT-β-R

columns essentially as described above for the expression and purification of human [**LT-α/â**] **LT-α/β** complexes. Armenian hamsters were immunized with purified soluble murine LT-α/ß complex essentially as described in Example 6. Hamster spleen cells were fused to the mouse P3X hybridoma cell line as described (Sanchez-Madrid et al., Methods of Enzymology, 121, pp. 239-44 (1986)). Hybridomas were grouped as [**anti-mLT-â**] **anti-mLT-β** or anti-mLT-α on the basis of their binding characteristics to either the [**LT-α/â**] **LT-α/β** complex or to LT-α alone, respectively. Hybridoma cells were expanded and the antibodies purified from the culture supernatant using Protein A affinity chromatography (Pharmacia).

Page 48, lines 10-22:

To assess whether hamster anti-mouse LT-á and LT-ß mAbs could block LT ligand binding to [m**LT-â-R**] **mLT-β-R**, we used TIMI-4 cells (ATCC), a murine T cell line that expresses surface LT ligand following PMA activation for 7 hours. Hamster anti-mLT-α or anti-mLT-ß mAbs were preincubated with the cells for 30 minutes at 4 C and then washed twice. The washed cells were incubated with 1 μg/ml of mLT-ß-R-Fc at 4 C. After 30 minutes, cells were washed free of unbound mLT-ß-R-Fc and then incubated for 30 minutes with 10 μg/ml of phycoerythrin-labelled donkey anti-human IgG to detect bound mLT-ß-R-Fc. The amount of bound fluorescent label was determined by FACS analysis and the mean fluorescence intensity was calculated.

Page 48, lines 23-26:

Using this analysis, it was found that the hamster [**anti-mLT-â mAb**] **anti-mLT-β mAb** could effectively block soluble LT-ß receptor binding to T cell surface LT ligand. The results are shown in Table 2.

REMARKS

The instant application is a divisional of U.S.S.N. 09/000,166. In reply to the Restriction Requirement mailed on June 20, 2000 in the 09/000,166 case Applicants elect prosecution of Group III, claims 54-67. In addition, entry of the above amendments to add continuing data and to correct a typographical error (the "β" symbol was

inadvertently listed as the "â" symbol) in the application is respectfully requested. As such no new matter is added by these amendments.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 02-2327**. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

If the Examiner believes that a telephone conference would expedite the prosecution of this application, please call the undersigned at (617)-679-2079.

Respectfully submitted,

Date: 2/14/02

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CLEAN COPY

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